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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

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The title of the application as originally filed reads as follows:
Cereal product containing probiotics

Field of the Invention

This invention relates to a ready-to-eat cereal product which contains a probiotic micro-organism; for example breakfast cereals, infant cereals or convenience foods. In use, the cereal product has a beneficial effect in the gastro-intestinal tract of the person consuming it and hence upon the person. The invention also relates to a process of producing the cereal product and to methods of promoting beneficial effects in the gastro-intestinal tracts of humans.

Background of the invention

Probiotic micro-organisms are micro-organisms which beneficially affect a host by improving its intestinal microbial balance (Fuller, R; 1989; J. Applied Bacteriology, 66: 365-378). In general, probiotic micro-organisms produce organic acids such as lactic acid and acetic acid which inhibit the growth of pathogenic bacteria such as *Clostridium perfringens* and *Helicobacter pylori*. Consequently, probiotic bacteria are believed to be useful in the treatment and prevention of conditions caused by pathogenic bacteria. Further, probiotic micro-organisms are believed to inhibit the growth and activity of putrefying bacteria and hence the production of toxic amine compounds. It is also believed that probiotic bacteria activate the immune function of the host.

Therefore there is considerable interest in including probiotic micro-organisms into foodstuffs. For example, many fermented milk products which contain probiotic micro-organisms are commercially available. Usually these products are in the form of yoghurts and an example is the LC1® yoghurt (Société des Produits Nestlé SA). Several infant and follow-up formulas which contain probiotic micro-organisms are also commercially available; for example the BIO NAN® formula (Société des Produits Nestlé SA).

However, there are two main issues in incorporating probiotic micro-organisms into foodstuffs. First, the foodstuff must be in a form which is palatable to a consumer. Secondly, the probiotic micro-organism must remain viable during storage. The second issue is particularly problematic for ready-to-eat cereal products. These cereal products, unlike fermented milks, are required to have long storage lives; for example at least a year while the cell counts for many probiotic micro-organisms may fall away completely within one or two days.

Therefore there is a need for a ready-to-eat cereal product which contains a probiotic micro-organism, is highly palatable, and which is storage stable.

Summary of the Invention

Accordingly, in one aspect, this invention provides a dried, ready-to-eat cereal product comprising a gelatinised starch matrix which includes a coating or filling containing a probiotic micro-organism.

It has been found that probiotic micro-organisms remain viable for extended periods of time when formulated into a coating on or filling in a dried cereal product. This is surprising since probiotic micro-organisms ordinarily die off rapidly. Therefore the invention offers the advantage of a ready-to-eat cereal product which is highly palatable and which contains a shelf stable source of probiotic micro-organisms.

The cereal product may be in the form of a breakfast cereal, an infant cereal, or a convenience food such as a cereal bar. Preferably, the gelatinised starch matrix is in flaked or expanded form. The gelatinised matrix is preferably produced by extrusion cooking a starch source.

Preferably the coating comprises a carrier substrate which carries the probiotic micro-organism in it. The filling may also comprise a carrier substrate which carries the probiotic micro-organism in it. For example, the carrier substrate may be fat, milk solids, sugar or a particulate flavouring agent.

In a further aspect, this invention provides a process of preparing a dried, ready-to-eat cereal product, the process comprising cooking a starch source to form a gelatinised starch matrix; forming the gelatinized starch matrix into pieces and drying the pieces; and coating or filling the pieces with a substrate which contains probiotic micro-organisms.

In one embodiment, the gelatinized starch matrix is formed into pieces and dried by extruding the gelatinised matrix to form a cooked extrudate and cutting and drying the cooked extrudate to form dried pieces. The gelatinised matrix may be caused to expand upon extrusion to form, after cutting and drying, expanded pieces. Alternatively, the pieces may be subjected to flaking to form flaked pieces.

In another embodiment, the gelatinized starch matrix may be formed into pieces and dried by roller-drying the gelatinised starch matrix to form flakes.

In a further embodiment, the gelatinized starch matrix may be formed into pieces and dried by extruding the gelatinised matrix to form a cooked extrudate containing an aperture; and cutting and drying the pieces. Preferably the gelatinised starch matrix is extruded with a central bore for receiving a filling.

Detailed description of preferred embodiments of the invention

Embodiments of the invention are now described, by way of example only. The invention provides a dried, ready-to-eat cereal product in the form of a gelatinised starch matrix which includes a coating or filling. The coating or filling contains a probiotic micro-organism. The probiotic micro-organism may be selected from one or more micro-organisms suitable for human consumption and which are able to improve the microbial balance in the human intestine.

Examples of suitable probiotic micro-organisms include yeasts such as *Saccharomyces*, *Debaromyces*, *Candida*, *Pichia* and *Torulopsis*, and bacteria such as the genera *Bifidobacterium*, *Bacteroides*, *Fusobacterium*, *Melissococcus*, *Propionibacterium*, *Streptococcus*, *Enterococcus*, *Lactococcus*, *Staphylococcus*, *Peptostreptococcus*, *Bacillus*, *Pediococcus*, *Micrococcus*, *Leuconostoc*, *Weissella*, *Aerococcus*, *Oenococcus* and *Lactobacillus*. Specific examples of suitable probiotic micro-organisms are: *Saccharomyces cerevisiae*, *Pediococcus acidilactici*, *Bacillus coagulans*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Enterococcus faecium*, *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, *Lactobacillus reuteri*, *Lactobacillus helveticus*, *Lactobacillus sake*, *Lactobacillus delbrückii* subsp. *lactis*, *Lactobacillus farciminus*, *Lactobacillus alimentarius*, *Lactobacillus casei* *Shirota*, *Lactobacillus casei* subsp. *casei*, *Lactobacillus gasseri*, *Lactobacillus curvatus*, *Lactococcus lactis*, *Lactobacillus rhamnosus* (*Lactobacillus* GG), *Lactobacillus reuteri*, *Lactobacillus helveticus*, *Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Enterococcus faecalis*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Pediococcus halophilus*, *Staphylococcus xylosus*, *Staphylococcus carnosus*, and *Micrococcus varians*. The probiotic micro-organisms are preferably in powdered, dried form; especially in spore form for micro-organisms which form spores. Further, if desired, the probiotic micro-organism may be encapsulated to further increase the probability of survival; for example in a sugar matrix, fat matrix or polysaccharide matrix.

The dried, ready-to-eat cereal product may be produced from any suitable ingredients; such as those commonly used in dried, ready-to-eat cereal products. One of these ingredients is a starch source. Suitable starch sources are, for example, grain flours such as corn, rice, wheat, barley, and oats. Also mixtures of these flours may be used. The flours may be whole flours or may be flours which have had fractions removed; for example the germ fraction or husk fraction may be removed. Rice flour, corn flour and wheat flour are particularly suitable; either alone or in combination. The starch source will be chosen largely on the basis of the nutritional value, palatability considerations, and the type of cereal product desired.

The cereal product may be produced in many different ways as desired. However, an especially suitable way of producing the cereal product is by extrusion cooking. This may be done as is well known in the art. For example, in one suitable process, a feed mixture is fed into a preconditioner. The feed mixture is primarily made up of the starch source and other ingredients such as sugar, salt, spices, seasonings, vitamins, minerals, flavouring agents, colouring agents, antioxidants, protein sources, fats and the like. Suitable protein sources are milk powders, whey powders, wheat glutens, etc. If desired, sources of insoluble fibre may also be included; for example wheat bran, corn bran, rice bran, rye bran and the like. Further, if desired, a source of soluble fibre may be included, for example, chicory fibres, inulin, fructooligosaccharides, soy oligosaccharides, oat bran concentrate, guar gum, carob bean gum, xanthan gum, and the like. Preferably the soluble fibre selected is a substrate for the micro-organism selected, or such that the soluble fibre and micro-organism form a symbiotic relationship for promoting beneficial effects. The maximum level of soluble fibre is preferably about 20% by weight; especially about 10% by weight.

Depending upon the desired form of the cereal product, the starch content of the feed mixture may be varied. For example, for an expanded cereal product, the feed mixture preferably includes up to about 40% by weight of starch. However, for a flaked product, it is not necessary to use large amounts of starch in the feed mixture since it is possible to flake an unexpanded product.

In the preconditioner, water or steam, or both, is mixed into the feed mixture. Sufficient water or steam is mixed into the feed mixture to moisten the feed mixture. If desired, the temperature of the feed mixture may be raised in the preconditioner to about 60°C to about 90°C by weight. A suitable

preconditioner is described in US patent 4,752,139. It is not necessary to subject the feed mixture to preconditioning but it is advantageous to do so.

The moistened feed leaving the preconditioner is then fed into an extruder. The extruder may be any suitable single or twin screw, cooking-extruder.

5 Suitable extruders may be obtained from Wenger Manufacturing Inc, Cletral SA, Bühler AG, and the like. During passage through the extruder, the moistened feed passes through a cooking zone, in which it is subjected to mechanical shear and is heated; for example up to a maximum temperature of up to about 150°C, and a forming zone. The gauge pressure in the forming zone is about 300 kPa to
10 about 10 MPa, as desired. If desired, water or steam, or both, may be introduced into the cooking zone. During passage through the extruder, the starch source of the moistened feed is gelatinised to provide a gelatinised starch matrix.

If desired, a small amount of an edible oil may be fed into the extruder along with the moistened feed to facilitate the extrusion process or as a carrier for
15 oil soluble additives. Any suitable oil may be used; for example vegetable oils such as sunflower oil, safflower oil, corn oil, and the like. If oils are used, oils which are high in mono-unsaturates are particularly preferred. Hydrogenated oils or fats are also preferred. The amount of oil used is preferably kept below about 1% by weight.

20 The gelatinised matrix leaving the extruder is forced through a suitable die; for example a die as described in European patent application 0665051; the disclosure of which is incorporated by reference. A shaped extrudate, which has a cross-sectional shape corresponding to that of the orifice of the die, leaves the die. If it is desired to produce a centred-filled cereal product, the gelatinised
25 matrix may be extruded with a central bore. The shaped extrudate is then cut into pieces using rotating blades at the exit of the die. Depending upon the conditions in the extruder and the composition of the shaped extrudate, the shaped extrudate expands to a greater or lesser extent.

30 If a flaked product is to be produced, the pieces may then be transferred to a flaking apparatus. Suitable apparatus are well known and widely used in the cereal industry and may be purchased from, for example, Bühler AG in Switzerland. If desired, the pieces may be partially dried before flaking.

The expanded or flaked pieces are then dried to a moisture content below about 5% by weight. This is conveniently carried out in a hot air drier as is
35 conventional. Moisture contents of about 1% to about 3% by weight are preferred. The expanded pieces produced in this way have a crispy, pleasant

texture and good organoleptic properties. The flaked pieces also have good texture and organoleptic properties. The pieces have a pleasant taste of toasted cereal. Conveniently, the density of the pieces may be less than about 300 g/l. At this point, the pieces usually have a water activity of about 0.15 to about 0.3.

5 The probiotic micro-organisms are then mixed into a suitable carrier substrate. Suitable carrier substrates include liquids, such as fats and sugar solutions, and particulate coatings such as particulate flavour coatings. Suitable fats are edible vegetable oils and fats; for example hydrogenated soy fat. Suitable particulate flavour coatings include sugars, chocolate powders, milk powders, 10 malted powders, flavoured beverage powders, and the like. If desired, the probiotic micro-organisms may be encapsulated. Protection agents to improve the survival of the micro-organisms may be incorporated into the carrier substrate. Examples of suitable protecting agents are vitamins such as vitamins C and E, amino acids and their salts such as lysine, glycine, cysteine and sodium 15 glutamate, sugars such as lactose, trehalose, saccharose, dextrine and maltodextrine, and proteins such as milk and soya proteins. Trace elements and minerals may also be included in the carrier substrate.

The selection of the carrier substrate will depend upon factors such as palatability considerations and the survival of the probiotic micro-organism since 20 some micro-organisms survive better in some carrier substrates than others. If fats are used in the carrier substrate, the carrier substrate preferably contains antioxidants to reduce the action of oxygen on sensitive micro-organisms. However selecting the optimum carrier substrate is a matter of simple trial and error for the skilled person. If necessary, the carrier substrate may be heated 25 slightly to melt it or to reduce its viscosity.

To produce a coated cereal product, any technique suitable for coating the pieces may be used. For example, in the case of a liquid carrier substrate, the mixture of the probiotic micro-organism and the carrier substrate may be sprayed onto the dried pieces. This may be carried out in any suitable manner. For 30 example, the pieces may be fed into a fluidized bed onto which the mixture is sprayed. Alternatively, the pieces may be fed into a rotary coater into which the mixture is sprayed. As a further alternative, the pieces may be caused to fall in a curtain and the coating mixture sprayed onto the curtain. In the case of a particulate carrier substrate, the probiotic micro-organism and the carrier 35 substrate may be mixed to form a dry mix. Heat sensitive components such as vitamins, amino acids, etc may also be included in the dry mix. The dry mix is

then agglomerated on the dried pieces using an agglomerating agent. A suitable procedure is described in US patent 4,777,056; the disclosure of which is incorporated by reference. Fats, oils and sugar solutions are examples of suitable agglomerating agents. Particulate carrier substrates may also be dusted onto the cereal product.

For a filled cereal product, the mixture of the probiotic and micro-organism and carrier substrate is filled into the central bore of each piece. In this case, the carrier substrate is preferably viscous or a substance which hardens rapidly. Fats are particularly suitable. Alternatively the cereal product and carrier substrate may be fed into a tumbler and the carrier substrate agglomerated to the cereal product using a syrup. In this case, the cereal product is coated and filled.

The dried, ready-to-eat cereal product conveniently contains about 10^4 to about 10^{10} cells of the probiotic micro-organism per gram of the dried cereal product; preferably about 10^6 to about 10^8 cells of the probiotic micro-organism per gram. The dried cereal product may contain about 0.5% to about 20% by weight of the mixture of the probiotic micro-organism and carrier substrate; preferably about 1% to about 6% by weight.

The dried cereal product may then be further processed as desired. For example, if the dried cereal is to be used as a breakfast cereal, dried fruit, nuts, other cereals, dried milk produce (such as dried yoghurt etc) may be dry mixed with or agglomerated with the coated cereal. If desired, the dried cereal may be further coated with protective agents or flavouring agents, or both. This may also be carried out prior to or during coating or filling of the dried pieces with the mixture of the probiotic and micro-organism and carrier substrate.

Alternatively the cereal product may be formulated into convenience foods such as snack bars and the like. Again the cereal product may be mixed with nuts, dried fruit, sugars or other sweeteners, colouring agents, or flavouring agents, and the like. A suitable binder, for example arabic gum or gelatine, may then be added. An agent which reduces breakability of the bar may also be included; for example hydrolysed wheat. If desired, the bar may be coated with a suitable coating; for example chocolate. Processes for manufacturing snack bars are well known and are described in the art; see for example US patent 4,871,557.

It will be appreciated that the dried, ready-to-eat cereal product may be produced by any suitable process and not only that described above.

The dried, ready-to-eat cereal product preferably comprises a nutritional supplement. When consumed in adequate amounts, the dried, ready-to-eat cereal

product results in a production of acids, such as lactic acid and acetic acid, in the gut of the consumer. This inhibits the growth of pathogenic bacteria or those which adversely affect well being, and has a beneficial effect on the consumer. Also, the probiotic micro-organisms adhere to intestinal surfaces and compete with undesired bacteria. Further, the growth and activity of putrefying bacteria may be inhibited and hence the production of toxic amine compounds. Adequate amounts of the dried, ready-to-eat cereal product may also result in the activation of the immune function of the consumer.

The amount of the dried, ready-to-eat cereal product to be consumed by the consumer to obtain a beneficial effect will depend upon the size and age of the consumer. However an amount of the dried, ready-to-eat cereal product to provide a daily amount of about 10^6 to about 10^{12} cells of the probiotic micro-organism would usually be adequate.

Numerous modifications may be made to the embodiments described above. For example, it is not necessary to produce the cereal product by extrusion cooking. Instead the cereal product may be produced by any suitable method of producing dried, ready-to-eat cereal pieces. For example, the feed materials may be cooked with water to provide a cooked paste. The paste is then roller-dried to produce dried flakes; usually of a thickness of about 0.6 to about 1 mm.

Specific examples are now described for further illustration.

Example 1

A feed mixture is made up of 70% by weight of corn flour, 17% by weight of wheat flour, 7% by weight sugar, 3% by weight of malt, 2% by weight of vegetable fats, and salt. The feed mixture is fed into a preconditioner and moistened. The moistened feed leaving the preconditioner is then fed into an extruder and gelatinised. The gelatinised matrix leaving the extruder is forced through a die and extruded. The extrudate expands upon leaving the die head and is cut into pieces of about 1 cm. The pieces are then dried to a moisture content of about 1% by weight.

The pieces are sprayed with two different coating mixtures. Each coating mixture contains sunflower oil as the carrier substrate but a different micro-organism. The micro-organisms are:

Micro-organism	Source	Form
<i>Saccharomyces cerevisiae</i>	Santel SA (Levucell™)	Spray dried powder
<i>Enterococcus faecium</i> SF68	Bioferment Division of Cerbios Pharma SA (LBC-K™), Switzerland	Spray dried powder

Both micro-organisms are commercially available. The pieces all contain about 10^6 cells/g to 10^7 cells/g of the probiotic micro-organism. To obtain an idea of the long term stability of the micro-organism, the pieces are stored at about 37°C. A sample of each group is taken immediately after production, after 1 week and 3 weeks.

The viable cell counts are determined for each sample. The results are as follows:

Micro-organism	Cell count - Day 1 (cells/g)	Cell count - 1 week (cells/g)	Cell count - 3 weeks (cells/g)
<i>Saccharomyces cerevisiae</i>	6.40×10^6	2.21×10^6	3.90×10^6
<i>Enterococcus faecium</i> SF68	1.38×10^6	8.60×10^6	4.03×10^6

The results indicate that the probiotic micro-organisms remain substantially stable.

Example 2

The procedure of example 1 is repeated except that the coating mixture is a dry mix of the probiotic micro-organisms and chocolate-flavoured powder (Nesquik® powder). The dry mix is coated on the pieces using the procedure described in US patent 4,777,056 and using vegetable oil as an agglomerating agent.

Further, the following micro-organisms are used:

Micro-organism	Source	Form
<i>B. coagulans</i>	Sankyo Pharmaceutical Company (Lacris-S TM), Japan	Powdered endospores
<i>L. johnsonii</i> La1	Nestec SA	Freeze dried powder
<i>Bifidobacterium animalis/longum</i>	Ch. Hansen A/S (Bb12 TM), Denmark	Freeze dried powder
<i>Saccharomyces cerevisiae</i>	Santel SA (Levucell TM)	Spray dried powder
<i>Enterococcus faecium</i> SF68	Bioferment Division of Cerbios Pharma SA (LBC-K TM), Switzerland	Spray dried powder

The first, third, fourth and fifth micro-organisms are commercially available. The second micro-organism is described in EP 0577904 and was deposited at the Collection Nationale de Cultures de Microorganismes (CNCM), Institut Pasteur, 28 rue du Dr Roux, 757724 Paris Cedex 15, France on 30 June 1992 under the number CNCM I-1225 and name La 1.

The cell counts determined for each sample. The results are as follows:

Micro-organism	Cell count - Day 1 (cells/g)	Cell count - 1 week (cells/g)	Cell count - 3 weeks (cells/g)
<i>B. coagulans</i>	6.37×10^6	5.07×10^6	4.24×10^6
<i>L. johnsonii</i> La1	1.43×10^6	3.21×10^5	1.39×10^5
<i>Bifidobacterium animalis/longum</i>	8.06×10^6	2.95×10^6	9.80×10^5
<i>Saccharomyces cerevisiae</i>	2.43×10^5	2.17×10^5	1.38×10^5
<i>Enterococcus faecium</i> SF68	1.94×10^6	5.70×10^5	1.50×10^4

The results indicate that the *B. coagulans* and *Bifidobacterium animalis/longum* are likely to remain stable for long periods. The other micro-organisms display less but acceptable stability.

Example 3

Expanded cereal products produced as described in example 1 are coated with three coating substrates. Product 1 is prepared by coating the cereal product with vegetable oil and then dusting on a spray-dried milk powder which contains *L. johnsonii* La1; Product 2 is prepared by coating the cereal product with vegetable oil and then dusting on a mixture of a spray-dried milk powder which contains *L. johnsonii* La1 and a cocoa-containing powder (Nesquik® powder); Product 3 is prepared by suspending a spray-dried milk powder which contains *L. johnsonii* La1 in a vegetable oil and spraying the oil (without pressure) on the cereal product.

The cell counts determined for each product. The results are as follows:

Product	Cell count - Day 1 (cells/g)	Cell count - 1 week (cells/g)	Cell count - 3 weeks (cells/g)
1	3.86×10^7	4.42×10^7	3.00×10^7
2	1.59×10^7	2.30×10^7	1.65×10^7
3	3.51×10^7	4.61×10^6	3.36×10^6

The results indicate that the probiotic micro-organisms remain substantially stable.

Example 4

A trial is conducted using 20 adult volunteers. Immediately prior to commencement of the trials, the gut flora of each volunteer is determined. The volunteers are then separated into two groups of 10 people. One group is fed, for breakfast, a 30 g portion of product 1 of example 3 along with cold milk. The other group is fed the same cereal product but without the coating of fat and probiotic micro-organism. Other meals during the day are the normal meals eaten by the volunteers.

After a week, the gut flora of each volunteer is analysed. The volunteer which are fed Product 1 have decreased counts of *C. perfringens*.

Claims

1. A dried, ready-to-eat cereal product comprising a gelatinised starch matrix which includes a coating or filling containing a probiotic micro-organism.
2. A cereal product according to claim 1 in the form of a breakfast cereal, an infant cereal, or a convenience food.
3. A cereal product according to claim 1 or claim 2 in which the gelatinised starch matrix is in flaked or expanded form.
4. A cereal product according to any of claims 1 to 3 in which the gelatinised matrix is an extrusion cooked starch source.
5. A cereal product according to any of claims 1 to 4 in which the coating or filling comprises a carrier substrate which contains the probiotic micro-organism.
6. A cereal product according to claim 5 in which the carrier substrate is a fat, milk solids, a sugar or a particulate flavouring agent.
7. A cereal product according to claim 4 further comprising a lipid layer on the gelatinized starch matrix, the lipid layer causing a particulate carrier substrate, which contains the probiotic micro-organism, to adhere to the gelatinized starch matrix.
8. A cereal product according to any of claims 1 to 7 in which the probiotic micro-organism is selected from *B. coagulans*, *L. johnsonii* La1, *Bifidobacterium animalis/longum*, *Saccharomyces cerevisiae*, and *Enterococcus faecium* SF68.
9. A cereal product according to any one of claims 1 to 8 further containing a source of soluble fibre.
10. A process of preparing a dried, ready-to-eat cereal product, the process comprising cooking a starch source to form a gelatinised starch matrix; forming the gelatinized starch matrix into pieces and drying the pieces; and coating or filling the pieces with a substrate which contains probiotic micro-organisms.